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REACTIVITY OF COMPRESSED ACETYLLYSINE-GLUCOSE-CELLULOSE MODELS

BY

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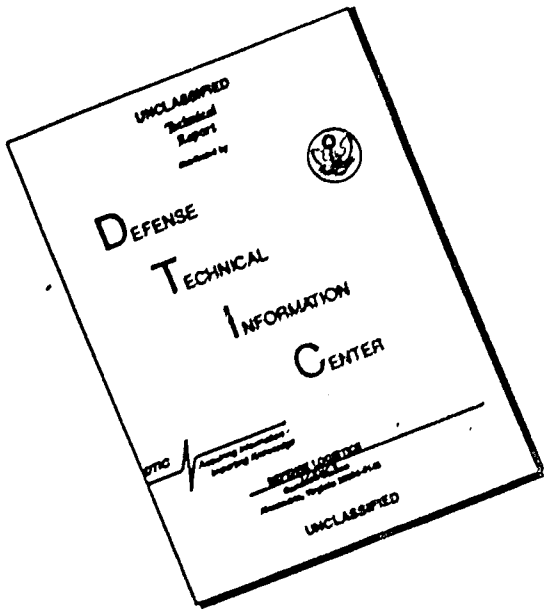

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The specific objectives of this investigation were: 1) to determine the quantitative aspects of lysine availability in a low moisture model containing acetyllysine, glucose and cellulose; 2) to compare the quality loss in acetyllysine as a result of interaction with glucose in compressed and uncompressed models; and 3) to compare the lysine-glucose-cellulose and acetyllysine-glucose-cellulose models with respect to lysine loss and changes in color and fluorescent emission characteristics. Compressed disks and uncompressed powders were stressed at 60°C and Aw = 0.23 for 0 to 144 hours. The quality loss of acetyllysine was determined indirectly by reducing capacity, furosine peak areas, glucose loss and directly by acetyllysine loss in these models. At 4 hours at 60°C and Aw = 0.23, the loss in acetyllysine was 39%. At 24 and 144 hours, the corresponding losses were 83 and 96% respectively. No accelerating or inhibiting effect of compression was noticed in the reaction between acetyllysine and glucose in contrast to a stabilizing effect upon lipid oxidation observed by Porter and Black. The acetyllysine-glucose-cellulose system was very reproducible

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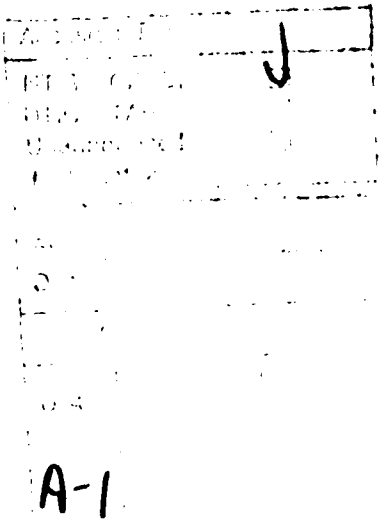
19. as measured by the losses in acetyllysine and glucose in two experiments conducted three months apart. Reducing capacity and furosine correlated exceedingly well with the loss in acetyllysine. Both fluorescence and color are not recommended as indicators for nutritionally available acetyllysine. The acetyllysine-glucose model gave rise to far less fluorescence and color compared with the earlier lysine-glucose-cellulose model. These data suggest that there is limited crosslinking in the acetyllysine-glucose-cellulose model compared with the lysine-glucose-cellulose model.



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PREFACE

Nutritional Sustainment Modules (NSMs) being developed for the battlefield conditions of the future (Army 21) require unprecedented energy density and extraordinary nutrient stability for prolonged periods (possibly up to one year) without refrigeration.

A recent observation at the U. S. Army Natick RD&E Center (project No. IL161102AH5203020) demonstrated that a compressed food model had increased oxidative stability of the fat component compared with its corresponding uncompressed food model. The nutritional quality of the protein component of foods is readily compromised under certain situations, e.g., intermediate water activity and the presence of reducing carbohydrates. It was therefore of interest to determine whether compression would also retard the loss in protein quality of food models as compared with uncompressed food models.

The present study (Project No. IL1611102AH5203018) was undertaken to compare the loss in the essential amino acid, lysine, in compressed and uncompressed acetyllysine-glucose-cellulose models. Additional information was sought regarding the use of other indicators, such as reducing capacity, furosine, fluorescence, and color, as a means of rapidly assessing losses in lysine. This study was undertaken during January 1985 to January 1987. A portion of this paper was presented at the Institute of Food Technologists Convention, June 15-18, 1986, Dallas, Texas.

ACKNOWLEDGEMENTS

We thank W. L. Porter, N. McCormick and M. Lightbody, U. S. Army Natick Research Development & Engineering Center (NRDEC) for their critical review of this manuscript. The secretarial assistance of P. Crawford and D. Zybas is gratefully acknowledged. We also thank R. Segars, NRDEC for his valuable help in the preparation of compressed models.

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INTRODUCTION

Among the many degradative interactions that take place in foods, the most difficult to arrest are those between proteins and reducing carbohydrates. This situation is even more aggravated when foods, such as combat rations of the future, are required to be stored for extended periods of time without refrigeration. Even when reducing carbohydrates are not part of product formulations, hydrolysis of the polysaccharides and oligosaccharides may occur during processing or during prolonged storage in the slightly acidic environment of most foods. Before solutions can be suggested to retard protein degradation, it is necessary to have a simple working model to assess the reactivity of lysine with reducing sugars under various optimum and adverse conditions.

It is well recognized that an amine, amino acid or a protein containing free amino groups can react (Fig. 1) with reducing sugars, through the Maillard reaction.[1] The Schiff base initially formed, cyclizes to the corresponding N-substituted glycosylamine and undergoes an irreversible Amadori rearrangement[1] to form a ketose sugar derivative (N-substituted 1-amino-1-deoxy, 2-ketose), commonly referred to as the Amadori compound. The Amadori compound is the end product of the initial stage of the Maillard reaction. The subsequent stages of the reaction give rise to a variety of fission products, carbonyl compounds, heterocyclic flavor compounds as well as brown melanoidin pigments.[1]

The Amadori compound as well as the carbonyl compounds formed have specific reducing properties, which make it possible to follow the course of the reaction. Acid hydrolysis (Fig. 2) of the Amadori compound gives rise to a unique protein quality indicator compound, furosine.[2]

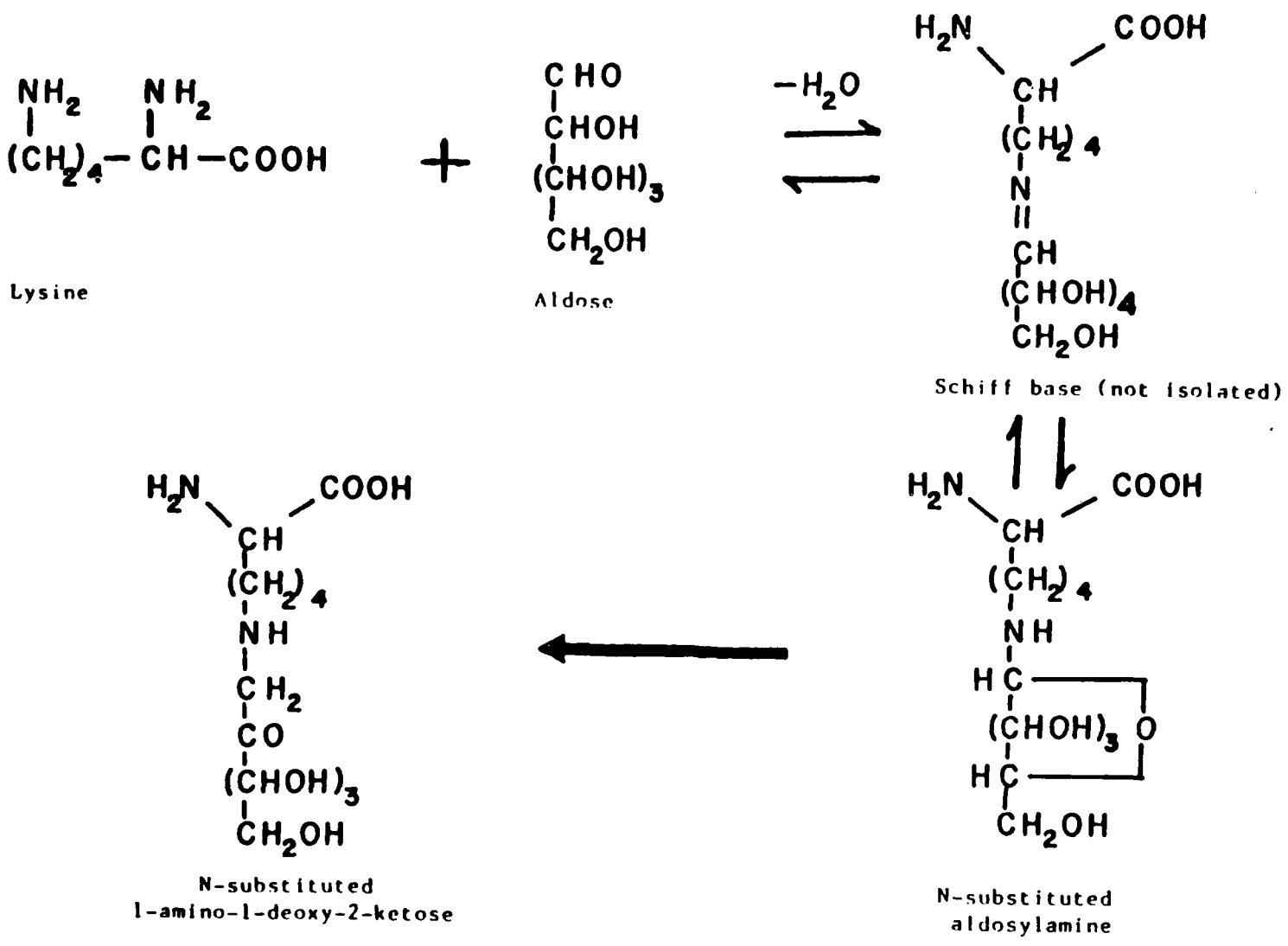

FIGURE 1.: SIMPLIFIED SCHEME OF THE EARLY PHASE OF THE MAILLARD REACTION.

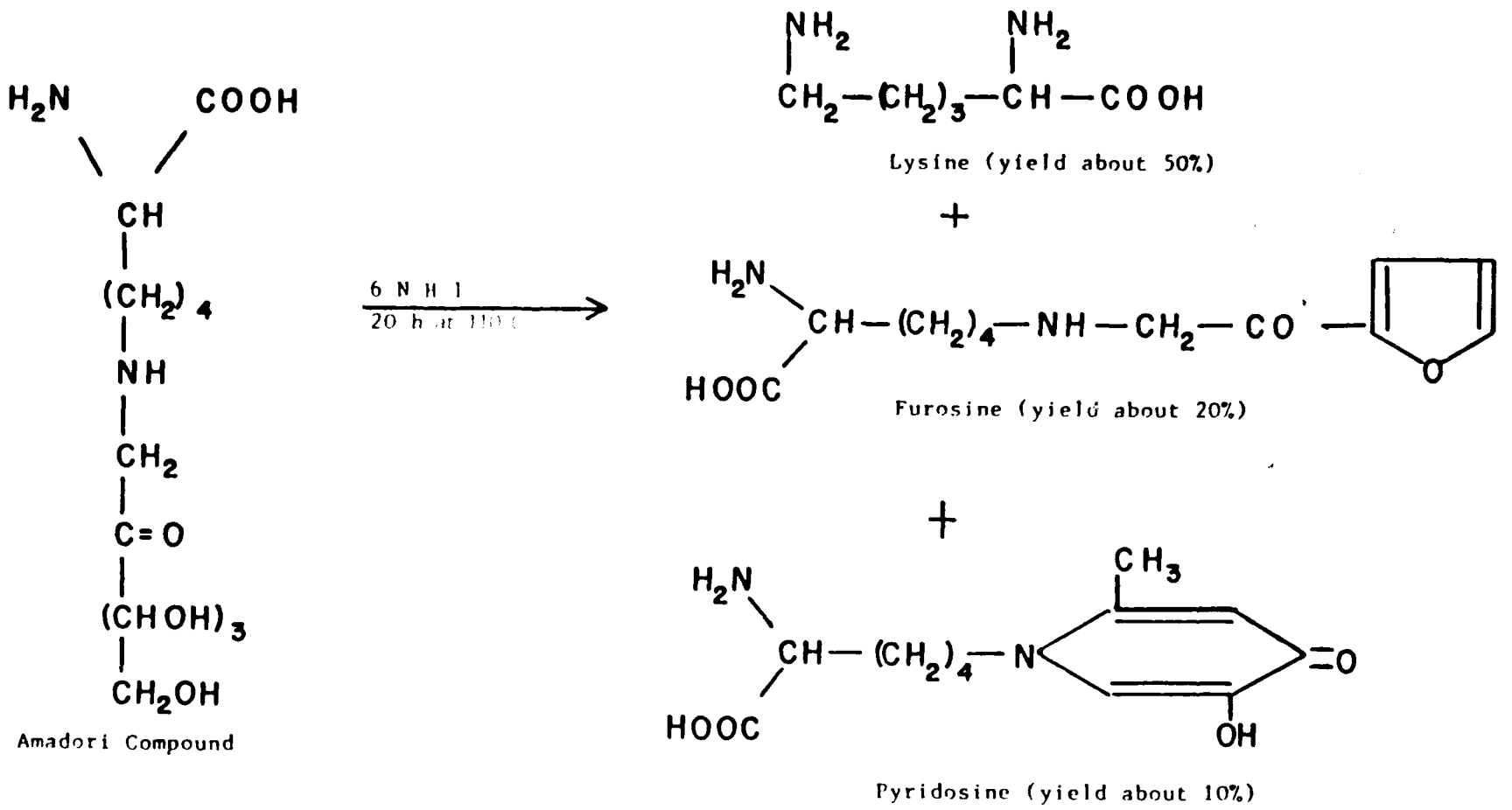

FIGURE 2.: FORMATION OF FUROSINE FROM AMADORI COMPOUND BY ACID HYDROLYSIS.

A large lag in lipid autooxidation in compressed, compared with uncompressed, food model systems, indicative of a beneficial effect of compression was recently observed by Natick researchers (W. L. Porter and E.D. Black).[3] Whether compression has a similar, beneficial effect upon Maillard degradative losses in the nutritional availability of lysine has not before been investigated. The specific objectives of this investigation were 1) to determine the quantitative aspects of lysine availability in a low-moisture model containing acetyllysine, glucose and cellulose; and 2) to compare the kinetics of the acetyllysine-glucose interaction in compressed and uncompressed models.

MATERIALS AND METHODS

Preparation of low moisture acetyllysine-glucose models. Separate N-$^{\alpha}$-acetyllysine (Sigma Chemical Co.) and glucose (Fisher Certified Reagent) solutions in highly purified deionized Milli Q water (Millipore Corp.) were prepared and the necessary volumes (usually 10 mL) to provide 5.4 mmoles (1 g) of acetyllysine and 12.2 mmoles (2.2 g) of glucose were pipetted into several freeze-drying flasks. Dispersions in Milli Q water containing 14.2 g of cellulose (Sigma 20, microcrystalline cellulose, 98% purity, <39 μ particle size, Sigma Chemical Co.) were then added to each flask, mixed well and immediately shell frozen in a alcohol-dry ice bath. The flasks were freeze-dried in a manifold-type freeze-dryer. The dried powders were mixed well in a blender and a portion was compressed into 2 g disks (4 mm thick, 25 mm diameter) using an Instron at 5500 psi. The freeze-dried powders (2 g samples) and the 2 g disks were incubated in separate glass jar incubators over saturated potassium acetate solution (a_w = 0.23) at 60^{o}C in a mechanical convection oven. At periodic

intervals, samples were withdrawn, cooled, and extracted in 0.05 M phosphate buffer pH 5.5 and analyzed for color, fluorescence characteristics, reducing capacity, furosine, glucose and N-α-acetyllysine.

Assay Procedures

Glucose was assayed by an enzymatic procedure by determining the reduction of NAD to NADH during phosphorylation of glucose to glucose-6-phosphate and its subsequent dehydrogenation to 6-phosphogluconate. Furosine was determined, following 6N HCL acid hydrolysis, at 280 nm with a Waters μBondapak C18 column and elution with an acetate buffer, pH 4.3.[4] Values are expressed as peak area units as obtained on a Waters Data Module 730 (Millipore Co, Milford, MA). Color was measured at 410 nm in a Bausch and Lomb spectrophotometer. Reducing power was measured by the reduction of ferricyanide in acidic pH.[5] Values are expressed as milliequivalents of reducing compounds based upon an ascorbic acid standard. Fluorescence intensity at the optimum excitation-emission wavelength (350 and 430 nm, respectively) was recorded with a Perkin Elmer-Hitachi Fluorescence spectrophotometer. Values are expressed as arbitrary units normalized to a quinine sulfate standard.

For the determination of acetyllysine, a modification of the recent picotag (Fig. 3) method described by Bidlingmeyer, Cohen and Tarvin[6] was utilized to separate the Amadori compound (bound acetyllysine) from the parent unreacted acetyllysine. A Waters (Waters Associates, Millipore Co., Milford, MA) High Performance Liquid Chromatography (HPLC) system, consisting of model 510 pumps, Waters Intelligent Sample Processor (model 710B), 721 System Controller, Kratos Absorbance Detector (Spectraflow 773) and a Waters Printer/Plotter/ Integrator (Data Module 730) were used. The

data for color, reducing capacity, furosine peak areas and fluorescence have been normalized to 1 mg NAL/mL at zero time for comparative purposes.

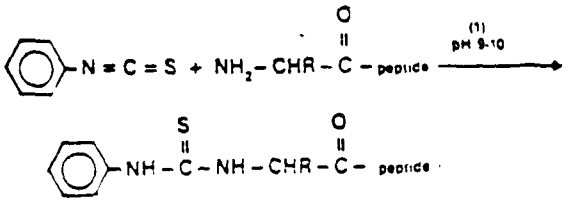

FIGURE 3.: PREPARATION OF PHENYLISOTHIOCARBAMYL (PITC) DERIVATIVES USING PHENYLISOTHIOCYANATE. PEPTIDE (OR AMINO ACID) AND ETHYL ALCOHOL-TRIETHYLAMINE-WATER (pH 9-10).

RESULTS AND DISCUSSION

Reactivity of N-α-Acetyllysine-Glucose-Cellulose (NAL-GL-CE) Models.

The degradation of N-α-acetyllysine (NAL) due to Maillard reaction with glucose (GL) in the acetyllysine-glucose-cellulose (NAL-GL-CE) system at 60°C and a_w = 0.23 was followed by monitoring: a) the production of furosine after acid hydrolysis; b) the increase in reducing capacity; c) the formation of fluorescent compounds; d) the increase in brown chromophoric components; e) the loss in NAL; and f) the decrease in GL.

The degradation of NAL and GL suggested an exponential decay, possibly first order kinetics (Figs. 4 and 5). Rate curves exhibiting exponential increases were observed for reducing power and furosine (Figs. 6 and 7). The fluorophoric and chromophoric compounds appeared to increase linearly (Figs. 8 and 9), at least until the end of the present experimental period.

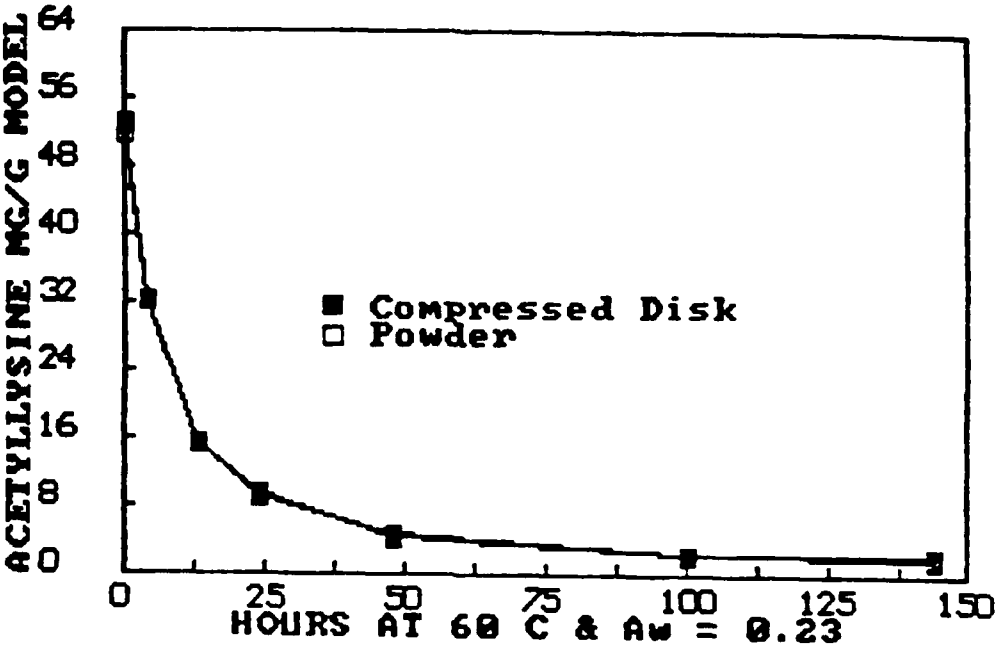

FIGURE 4. DEGRADATION OF ACETYLLYSINE IN COMPRESSED AND UNCOMPRESSED ACETYLLYSINE-GLUCOSE-CELLULOSE MODELS.

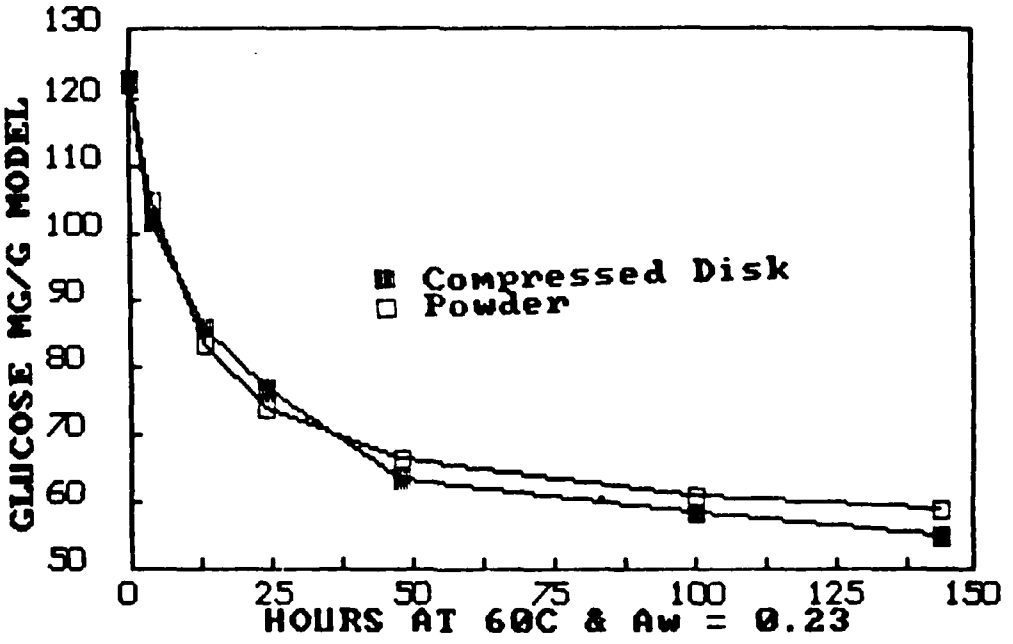

FIGURE 5. DEGRADATION OF GLUCOSE IN COMPRESSED AND UNCOMPRESSED ACETYLLYSINE-GLUCOSE-CELLULOSE MODELS.

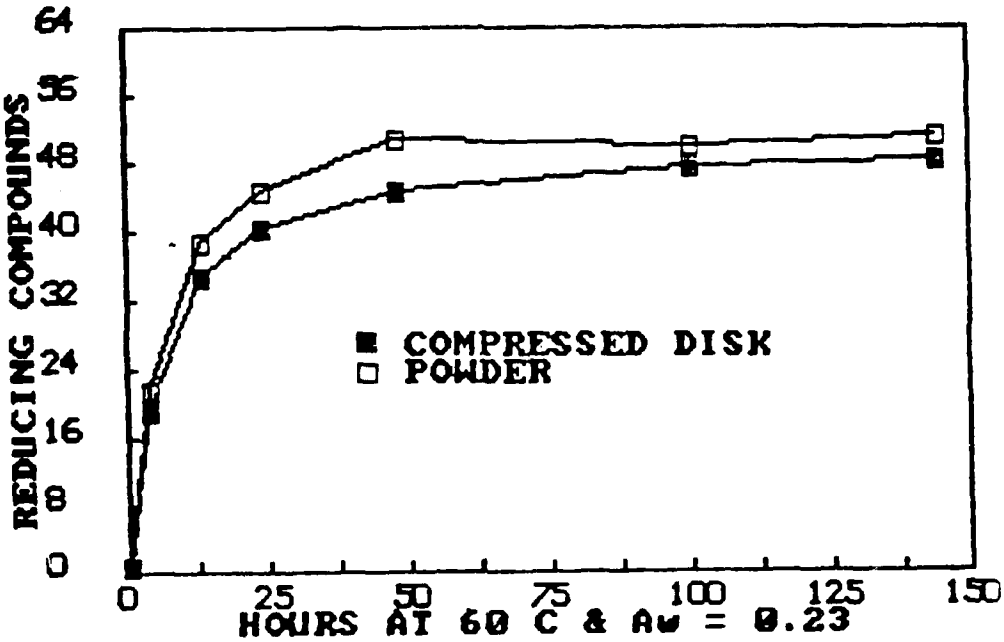

FIGURE 6. THE EFFECT OF INCUBATION ON THE FORMATION OF REDUCING COMPOUNDS (10^{-4} MILLIEQUIVALENTS/MG NAL) IN COMPRESSED AND UNCOMPRESSED ACETYLLYSINE-GLUCOSE-CELLULOSE MODELS.

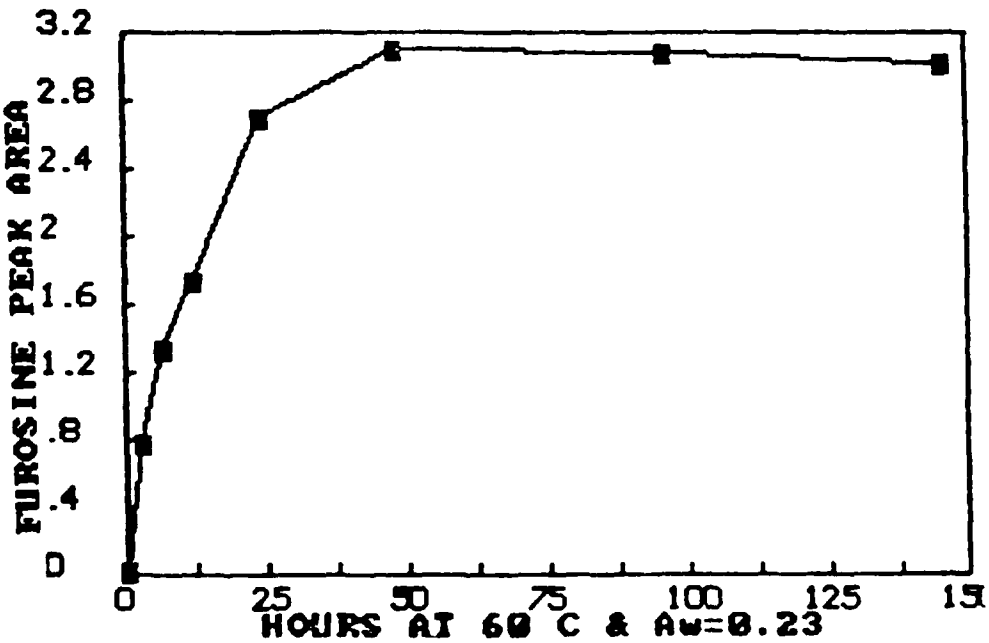

FIGURE 7. THE FORMATION OF AMADORI COMPOUND, AS ASSESSED INDIRECTLY BY 10^9 AREA UNITS OF FUROSINE PEAK/MG NAL IN THE COMPRESSED ACETYLLYSINE-GLUCOSE-CELLULOSE MODELS.

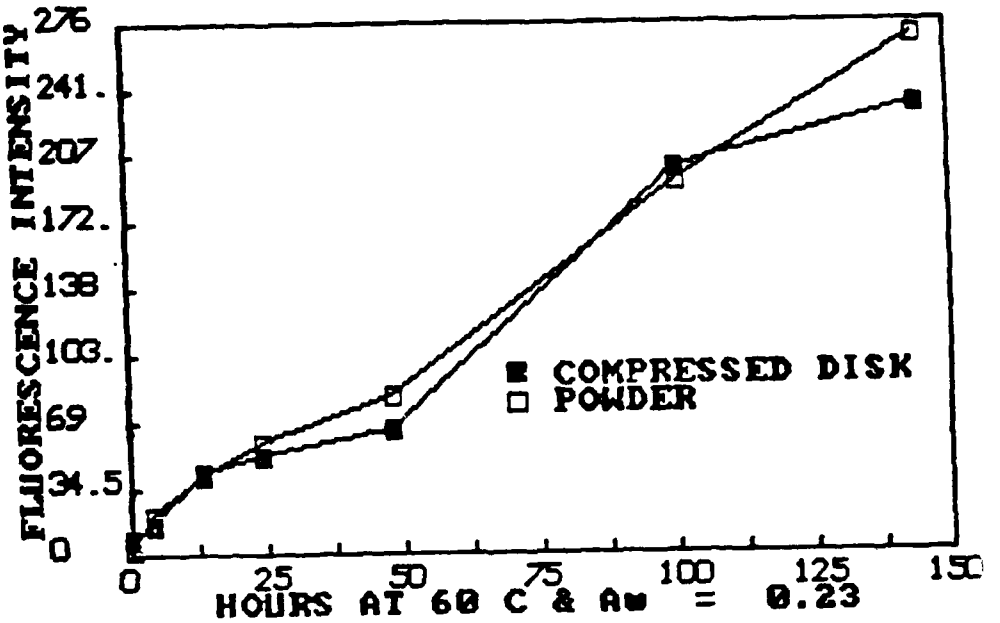

FIGURE 8. THE FORMATION OF FLUORESCENT COMPOUNDS (AS STANDARDIZED ARBITRARY UNITS/MG NAL) IN COMPRESSED AND UNCOMPRESSED ACETYLLYSINE-GLUCOSE-CELLULOSE MODELS.

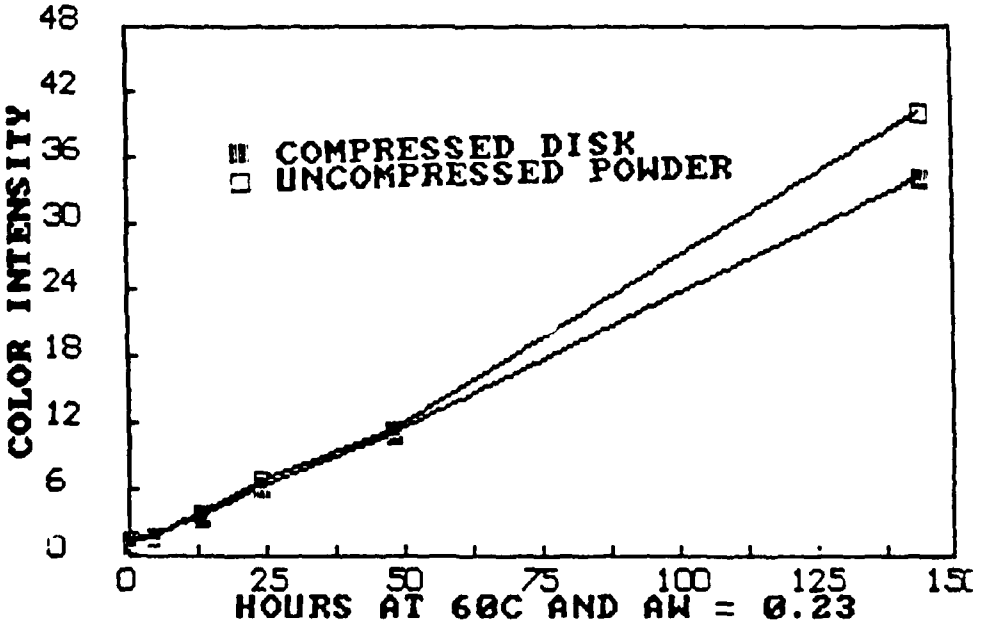

FIGURE 9. FORMATION OF COLORED COMPOUNDS, EXPRESSED AS (10^{-2} ABSORBANCE AT 410 NM)/MG NAL IN COMPRESSED AND UNCOMPRESSED ACETYLLYSINE-GLUCOSE-CELLULOSE MODELS.

Nutritional availability of acetyllysine due to Maillard reaction. The data indicated a decrease in NAL of 39% in 4 h at 60°C and a water activity of 0.23. Under a more realistic condition of storage at 40°C, previous data obtained with a lysine-glucose-cellulose model indicated that 25% of the lysine was lost in 144 hours at a_w = 0.23.[7] The losses at a_w = 0.23 in lysine at the end of 4 hours at 60°C and 40°C were 70% and 8%, respectively. At 144 hours, the loss in NAL at 60°C and a_w=0.23 was 96%. The moisture content of the NAL-GL-CE system was 2.0% as determined by prolonged dessication over phosphorous pentoxide. While the compromise of essential amino acids, particularly lysine, due to Maillard reaction is well known in intermediate moisture foods, it is not widely recognized that significant losses in lysine can occur at low water activity conditions. Although the degradation of NAL due to reaction with GL at 40°C and a_w = 0.23 in 144 hours is not yet established, it is likely to be significant on the basis of comparative data for lysine.

Reproducibility of the NAL-GL reaction in compressed systems. To assess the reproducibility of the experiment and procedures employed, the loss of NAL and GL were determined in two totally separate experiments, conducted three months apart using compressed NAL-GL-CE models. It is clear from both the glucose and lysine degradation curves (Figs. 10 and 11) that the reaction is surprisingly reproducible under these unusual conditions where the interaction is taking place in the solid state at very low water activity. Furthermore, the substantial loss of NAL of 83% in 24 hours at 60°C is confirmed.

Effect of compression. This study was undertaken to determine the effect of compression because of an observation by Natick researchers (W. L. Porter and E. D. Black)[3] of a greatly increased lag period (100 h) in

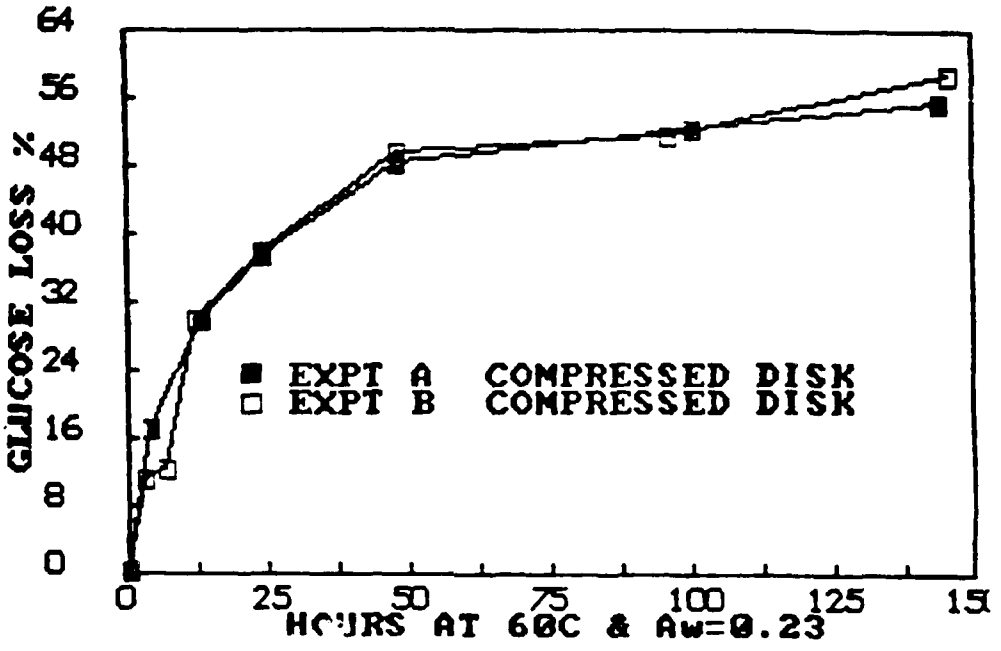

FIGURE 10. REPRODUCIBILITY OF GLUCOSE LOSS IN TWO SEPARATE EXPERIMENTS CONDUCTED THREE MONTHS APART WITH COMPRESSED ACETYLLYSINE-GLUCOSE MODELS.

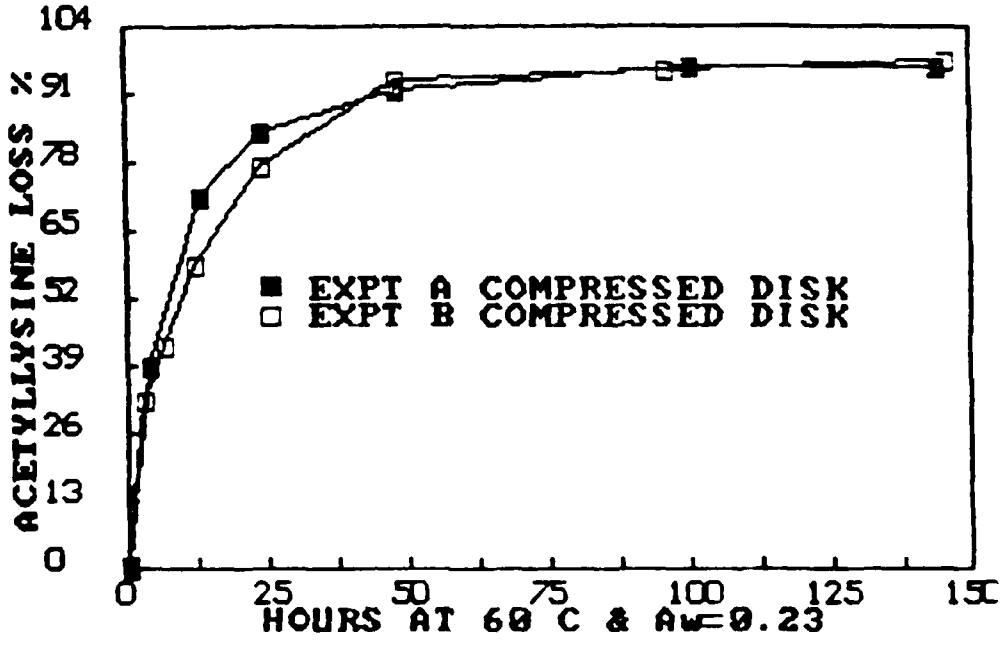

FIGURE 11. REPRODUCIBILITY OF ACETYLLYSINE LOSS IN TWO SEPARATE EXPERIMENTS CONDUCTED THREE MONTHS APART WITH COMPRESSED ACETYLLYSINE-GLUCOSE-CELLULOSE MODELS

lipid oxidation at 65°C with a compressed model compared with its corresponding uncompressed model (Fig. 12). The data (Figs. 4, 5, 6, 8, 9) obtained with compressed and uncompressed NAL-GL-CE models using several different parameters have clearly demonstrated that the rate curves of the acetyllysine-glucose reaction were essentially the same in compressed disks as in uncompressed powders. The absence of significant differences between the two models (compressed and uncompressed) would also appear to suggest that there is no major problem with respect to equilibration of the highly compressed system with the water activity of saturated potassium acetate in the jar. The kinetic data on NAL loss in compressed and uncompressed systems fitted exponential regression lines with slopes and intercepts matching within 4% and 1%, respectively. Therefore, these data together with the rate curves indicated that compression to 5500 psi has neither a beneficial nor a deleterious effect upon the stability of NAL in the presence of glucose. It may be tentatively inferred that compression per se may have no deleterious effect upon the lysine quality of compressed foods, such as dairy bars, which are important constituents of NSMs.

Correlations between acetyllysine loss and the alterations in other parameters. The concentration of reducing intermediates correlated almost perfectly ($r = 0.999$) with the loss in NAL (Fig. 13). Furosine peak areas also correlated extremely well ($r = 0.993$) with the loss in NAL (Fig. 14). There is a poor correlation between fluorescence intensity and the loss in NAL (Fig. 15). The emission intensity is very weak until almost 85% of the NAL is consumed. Hence, fluorescence is not recommended as a reliable indicator for predicting lysine losses.

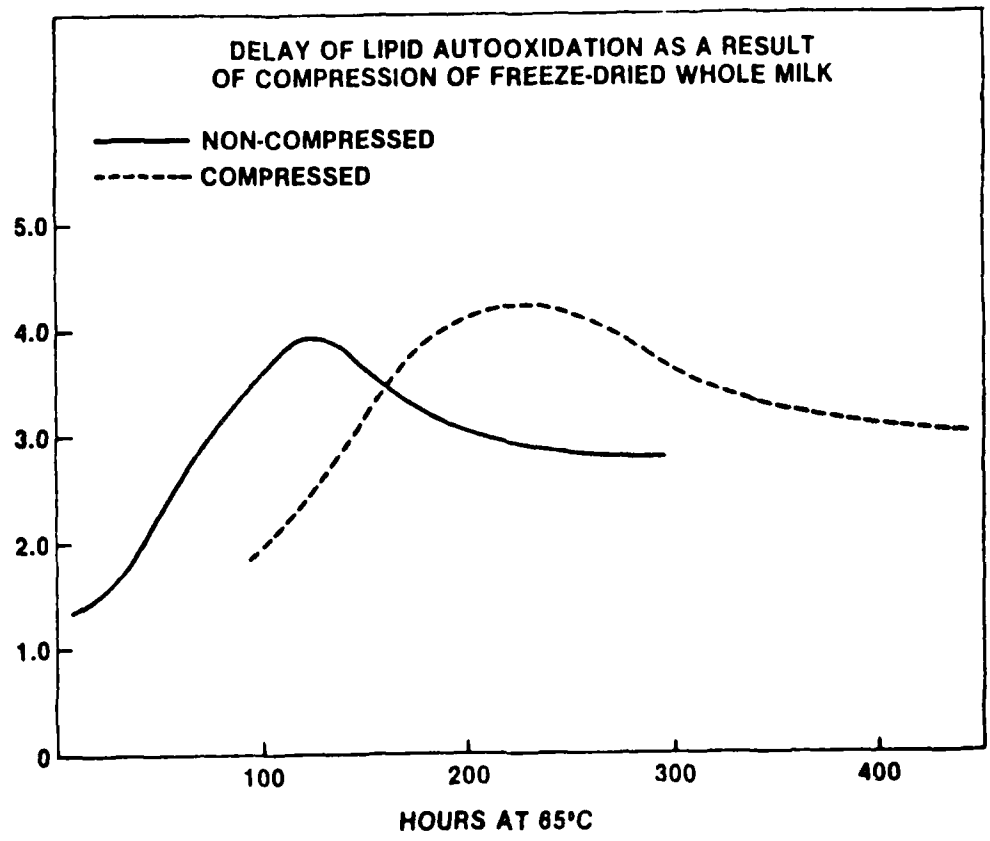

FIGURE 12. THE EFFECT OF COMPRESSION OF FREEZE-DRIED WHOLE MILK UPON THE LAG PERIOD OF LIPID AUTOOXIDATION. REPRODUCED WITH THE PERMISSION OF DR. PORTER AND DR. BLACK (REF. 3).

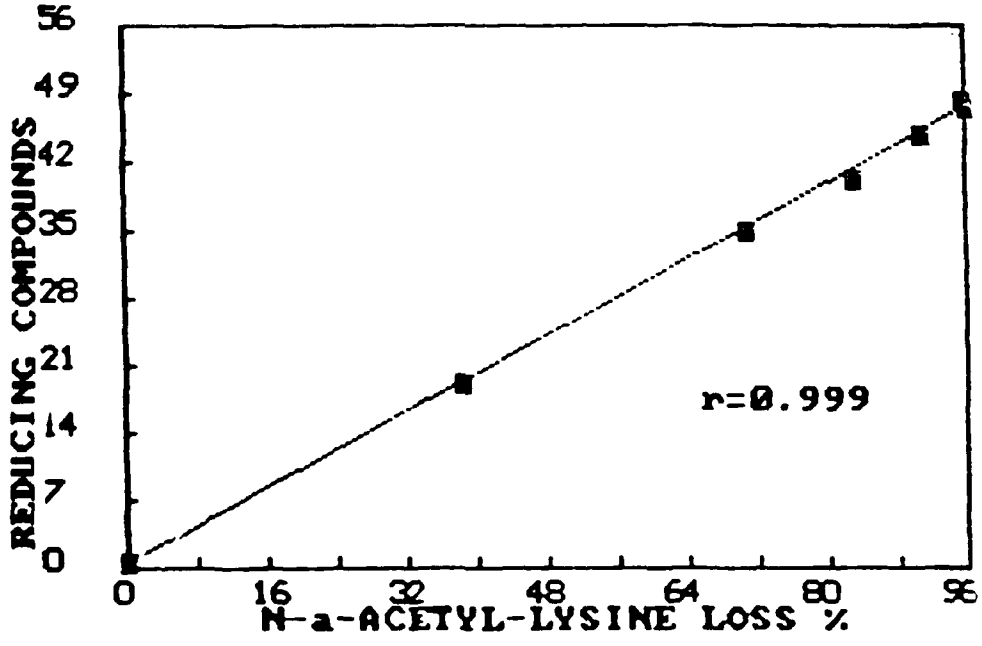

FIGURE 13. THE CORRELATION BETWEEN THE LOSS IN ACETYLLYSINE AND THE INCREASE IN REDUCING COMPOUNDS (10^{-4} MILLIEQUIVALENTS/MG NAL) WITH TIME IN THE COMPRESSED ACETYLLYSINE GLUCOSE-CELLULOSE MODEL AT 60°C AND Aw = 0.23.

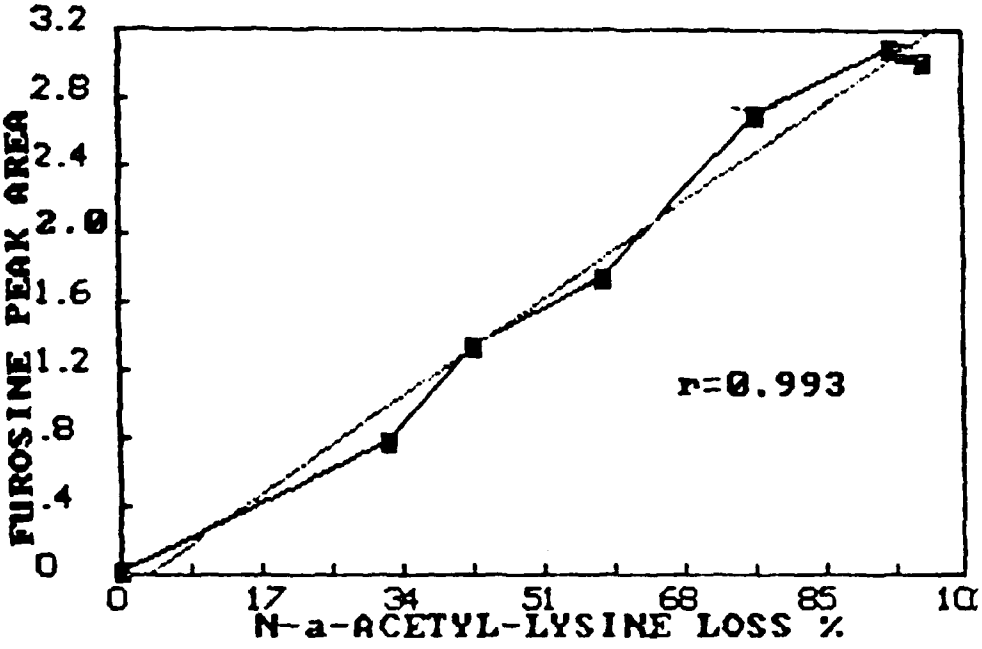

FIGURE 14. THE CORRELATION BETWEEN THE LOSS IN ACETYLLYSINE AND THE INCREASE IN FUROSINE (10^9 PEAK AREA UNITS/MG NAL) WITH TIME IN THE COMPRESSED ACETYLLYSINE-GLUCOSE-CELLULOSE MODEL AT 60°C AND Aw = 0.23.

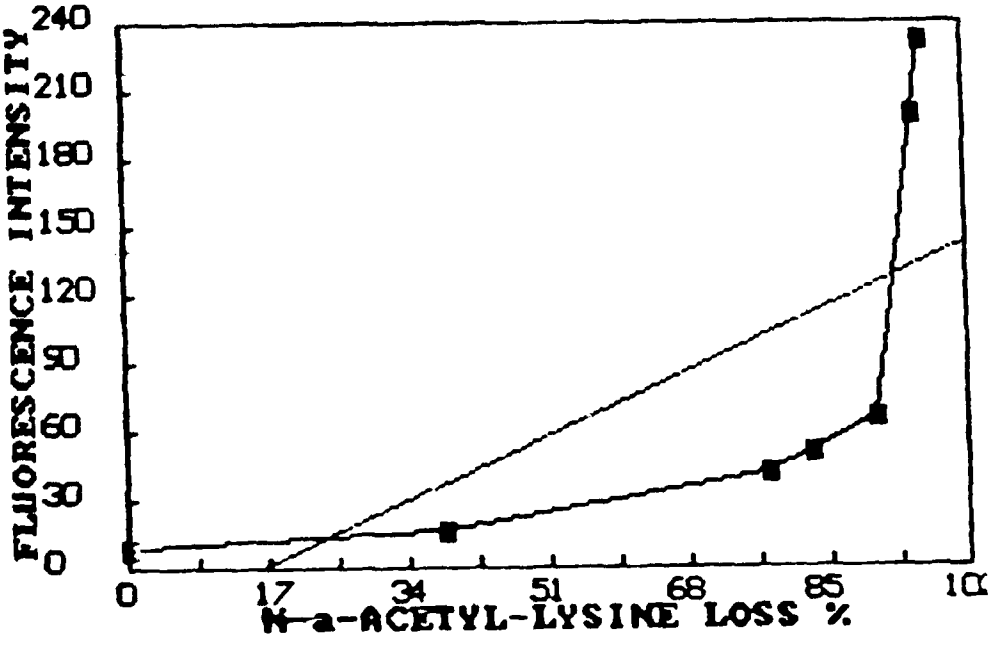

FIGURE 15. THE LACK OF CORRELATION BY LINEAR REGRESSION BETWEEN THE LOSS IN ACETYLLYSINE AND THE INCREASE OF FLUORESCENCE (STANDARDIZED ARBITRARY UNITS/MG NAL) WITH TIME IN THE COMPRESSED ACETYLLYSINE-GLUCOSE-CELLULOSE MODEL AT 60°C AND Aw = 0.23.

The correlation between color and the loss in NAL (Fig. 16) is even worse than that for fluorescence. There is very little change in absorbance until about 90% of the NAL is lost. Hence, color, which is commonly used as a quick assessment of the loss in protein quality by many food technologists, is an extremely poor indicator. If other interfering compounds are absent, color and fluorescence may provide an approximate estimate of lysine quality where the damage is below 60% Even here, it should be recognized that large inaccuracies can result because of extremely small slopes (Fig. 15 & 16)

Kinetics of the NAL-GL reaction in the solid state. Since the shape of the rate curve seemed to suggest first order kinetics, semilog plots of NAL and GL vs. time were obtained. It is evident that a straight-line relationship is not obtained (Fig. 17) between log (GL) and time. This suggests that the reaction does not follow first order kinetics. A similar conclusion was derived from the log (NAL) vs. time curve (Fig. 18). These data confirm previous observations with a lysine-glucose-cellulose system.[7,8] The halftime values estimated for various time spans of the present experiment are shown in Table 1. The t 1/2 estimated from the linear portion of the log (NAL)-time plot gave a value of 7.5 to 9.5 h which compared well with the estimate from the rate curve of about 9 h.

As we include more and more data points, the rate constant decreases and, consequently, the t 1/2 value increases. The regression coefficient is good only up to the 4th data point (at 24 h, when 92% of NAL had been exhausted). The deviation from a linear semilog concentration-time relationship has been previously observed by others[9,10] and by us with lysine-glucose models.[7,8]

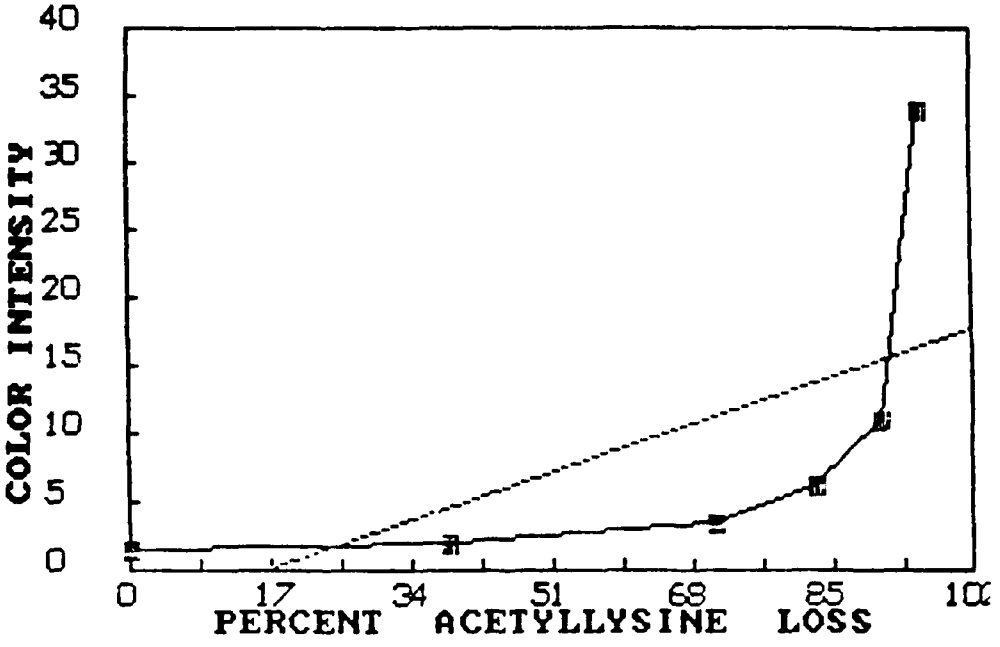

FIGURE 16. THE LACK OF CORRELATION BY LINEAR REGRESSION BETWEEN THE LOSS IN ACETYLLYSINE AND THE INCREASE IN COLOR (10^{-2} ABSORBANCE AT 410 NM/MG NAL) WITH TIME IN THE COMPRESSED ACETYLLYSINE-GLUCOSE-CELLULOSE MODEL AT 60°C AND Aw = 0.23.

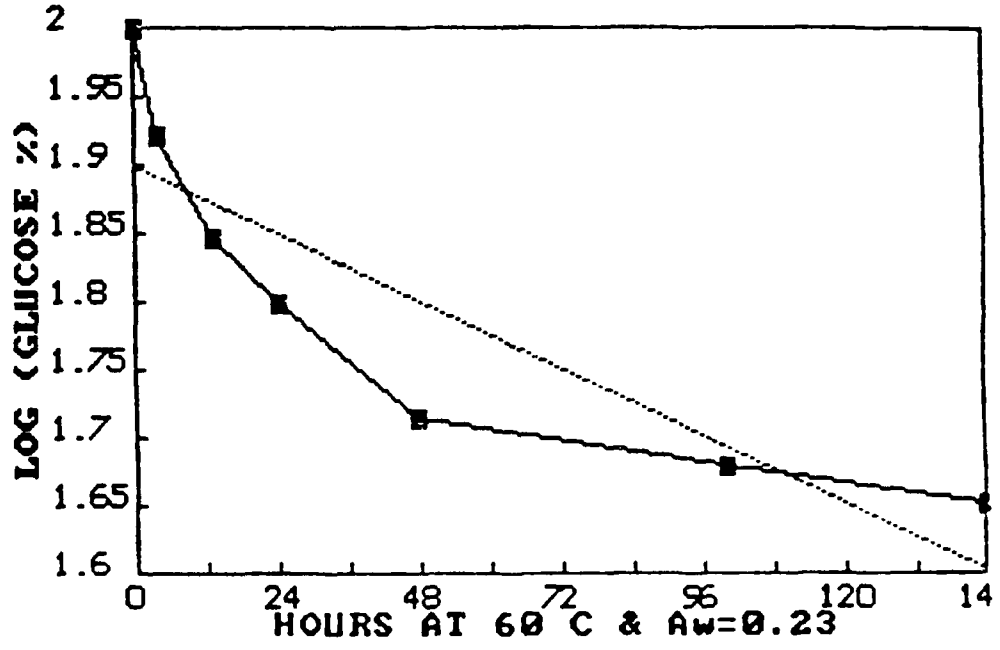

FIGURE 17. SEMILOG PLOT OF GLUCOSE CONCENTRATION (EXPRESSED AS PER CENT INITIAL) VS TIME IN THE COMPRESSED ACETYLLYSINE-GLUCOSE-CELLULOSE MODEL.

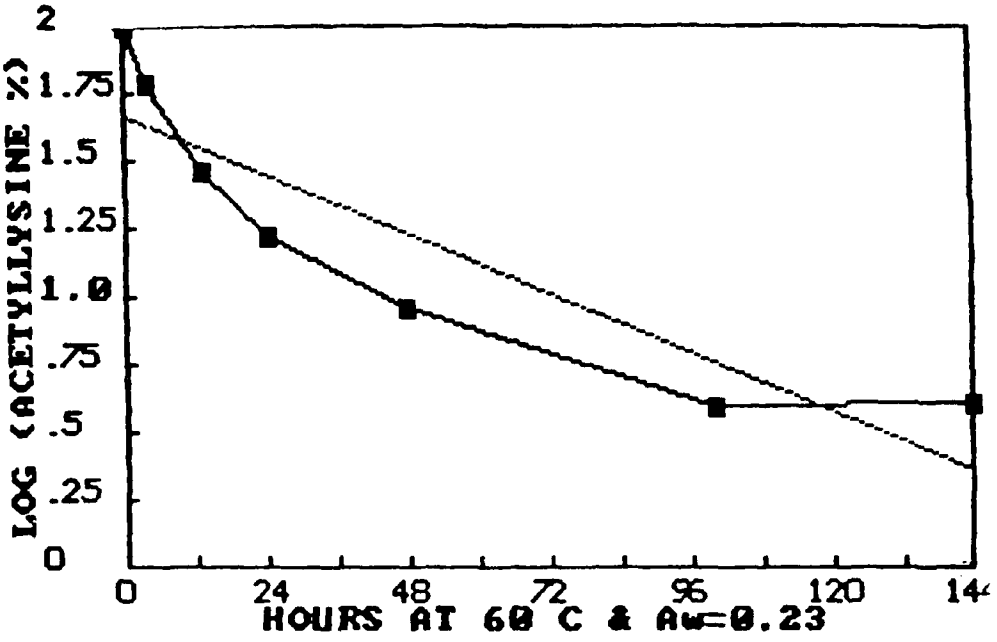

FIGURE 18. SEMILOG PLOT OF ACETYLLYSINE CONCENTRATION (EXPRESSED AS PER CENT INITIAL) VS TIME IN THE COMPRESSED ACETYLLYSINE-GLUCOSE-CELLULOSE MODEL.

TABLE I. First Order Rate Constants and Estimated First Order Half-times of NAL loss at 60°C

TIME INTERVAL HR	NO. OF DATA POINTS	REGRESSION COEFFICIENT	RATE CONSTANT (HR^{-1})	T 1/2 HR COMPUTED	T 1/2 HR OBSERVED
0-13.1	3	-0.995	0.0928	7.5	9.0
0-24.1	4	-0.985	0.0730	9.5	9.0
0-48.1	5	-0.954	0.0480	14	9.0
0-100	6	-0.930	0.0295	23	9.0
0-144	7	-0.896	0.0208	33	9.0

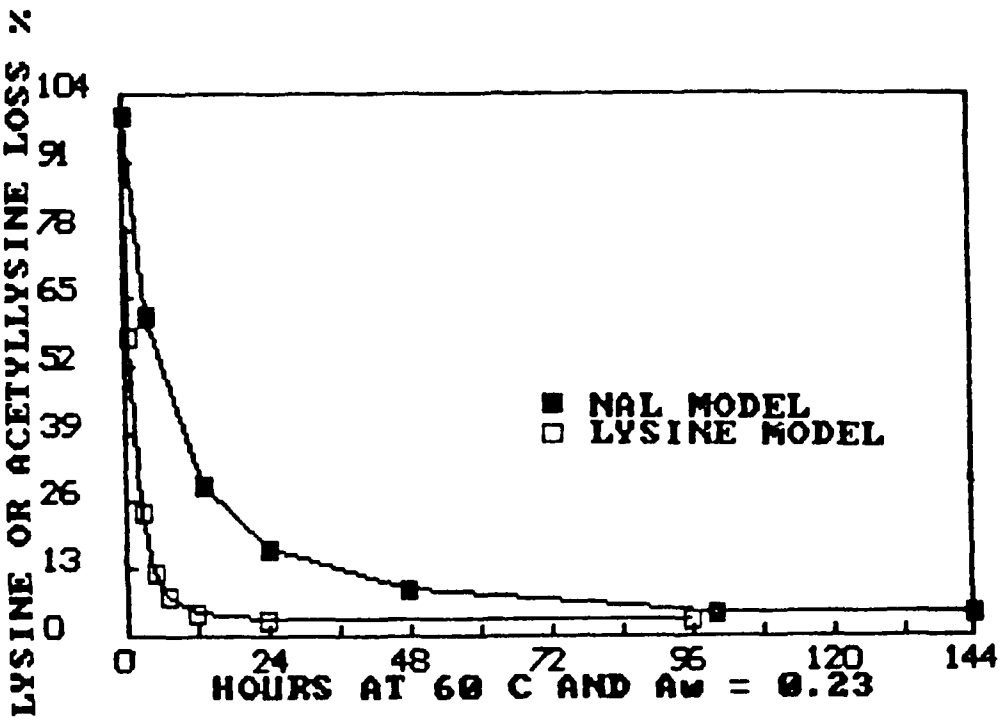

FIGURE 19. A COMPARISON OF THE RELATIVE LOSSES OF ACETYLLYSINE AND LYSINE IN ACETYLLYSINE-GLUCOSE-CELLULOSE AND THE LYSINE-GLUCOSE-CELLULOSE MODELS.

Comparison between lysine-glucose-cellulose and acetyllysine-glucose-cellulose models. Previous work from this laboratory was focused upon lysine-glucose models. Because of its single free ε-amino group, NAL has been used in the present study and it better represents the complex protein system in the food than does the lysine-glucose model where both & amino groups are free. Under the same experimental conditions of 60°C and a_w of 0.23, lysine is exhausted more rapidly (t 1/2 of 1.5 h) than is NAL (t 1/2 of 9 h). However, the differences in the lysine and NAL rate curves (Fig. 19) seen are not as vast as in the case of fluorescence or color (Figs. 20 and 21). In comparison with the NAL model, the color and fluorescence exhibit a dramatic enhancement in the lysine model. Lysine has two reactive amino groups unlike NAL, which has only one similar to the case of peptide-bound lysine in proteins. Consequently, the enhanced fluorescence and color is most likely due to greater reactivity and greater degree of cross-linking of free lysine compared to NAL.

In conclusion, a high degree of compression at 5500 psi failed to produce accelerating or inhibitory effects upon the NAL-GL reaction. The reaction at 60°C and a_w = 0.23 deviated significantly from first order kinetics. Both fluorescence and color were dramatically decreased in the NAL-GL compared with the lysine-GL system, suggesting that cross-linking was greatly minimized in the NAL-GL system. Studies under way with protein-glucose models may present a different picture compared with the NAL-GL model because of increased molecular size and the presence of other amino acids, including end amino acids with free amino groups. Further, amino acids adjacent to lysine residues may not be biologically available due to steric hindrance in enzyme catalysis.

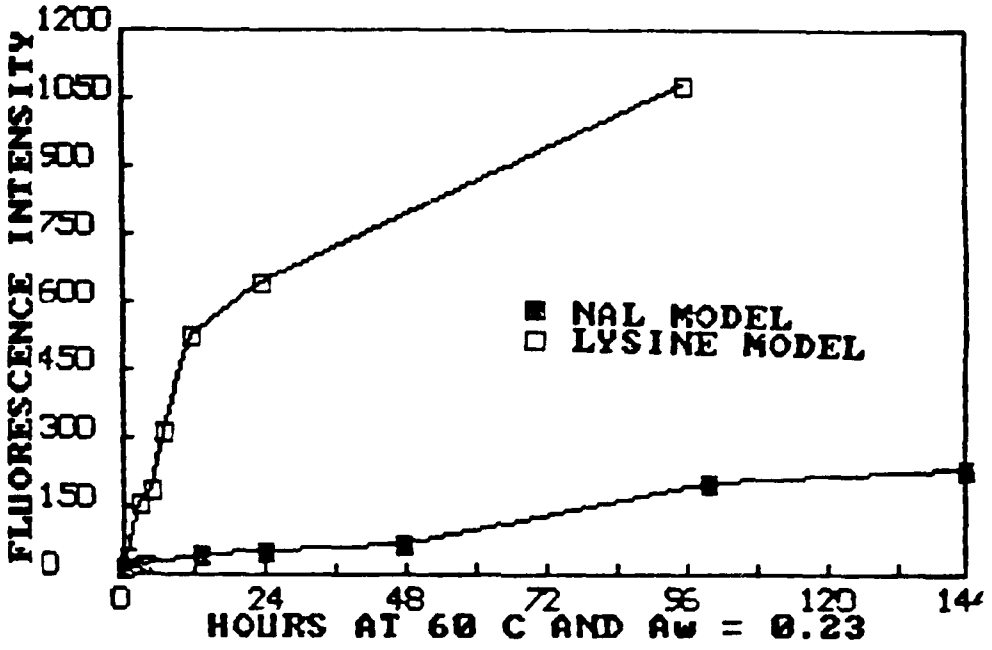

FIGURE 20. A COMPARISON OF THE RELATIVE CHANGES IN FLUORESCENCE INTENSITY EXPRESSED AS QUININE SULFATE STANDARDIZED UNITS/MG NAL OR LYSINE IN ACETYLLYSINE-GLUCOSE-CELLULOSE AND LYSINE-GLUCOSE-CELLULOSE MODELS.

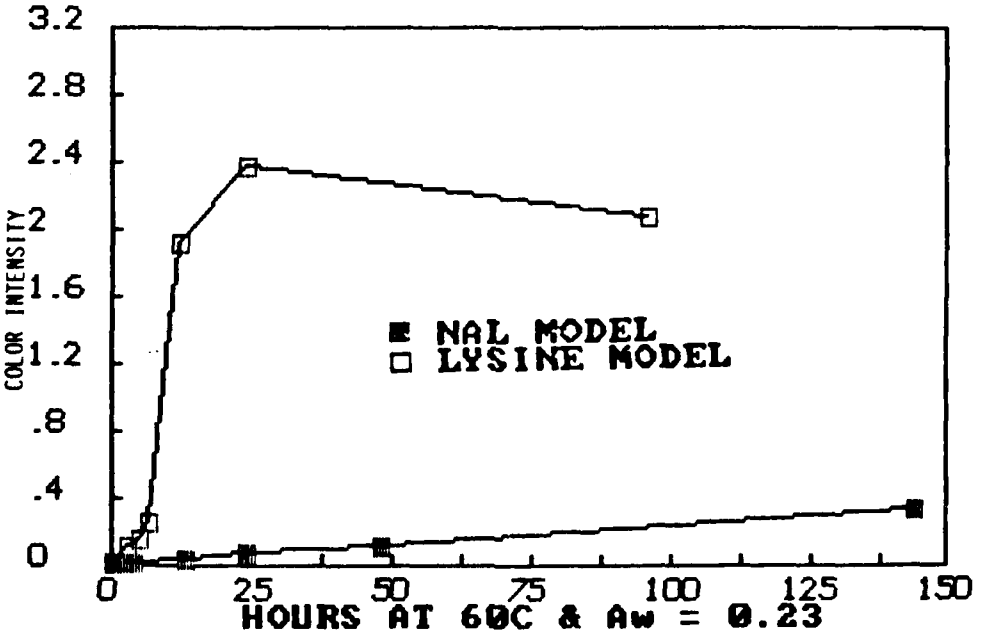

FIGURE 21. A COMPARISON OF THE RELATIVE CHANGES IN THE INTENSITY OF COLOR (ABSORBANCE AT 410 NM/MG NAL OR LYSINE) IN THE ACETYLLYSINE-GLUCOSE-CELLULOSE AND LYSINE-GLUCOSE-CELLULOSE MODELS.

CONCLUSIONS

Compression of a model system, acetyllysine-glucose-cellulose, did not decrease or increase the reaction between acetyllysine and glucose when compared with an identical uncompressed model. Hence compressed foods are not likely to undergo greater loss in protein quality than are uncompressed foods. As noted, beneficial effect due to compression on lipid autooxidation was recently demonstrated by Natick researchers (W. L. Porter and E. D. Black). Thus, significant differences exist between Maillard reaction and autooxidation in relation to the effect of compression.

The acetyllysine-glucose-cellulose system was very reproducible in two experiments conducted three months apart as measured by losses in acetyllysine and glucose with time.

Reducing capacity and furosine correlated exceedingly well with the loss in NAL. Both fluorescence and color are not recommended as reliable indicators for the loss in NAL because their intensities are extremely low below 70% loss in NAL. Hence large inaccuracies in data are predictable.

Acetyllysine is less reactive than lysine at 60°C and a_w = 0.23. Hence food proteins in the presence of reducing sugars are likely to be less reactive than the lysine-glucose system.

The acetyllysine-glucose-cellulose model gave rise to far less fluorescence and color compared with a lysine-glucose-cellulose model. These data suggest limited cross-linking in the acetyllysine-glucose-cellulose models compared with the lysine-glucose-cellulose models.

The loss in acetyllysine in 4 hours at 60°C and a_w = 0.23 was 39%.

RECOMMENDATIONS

From this study the following recommendations are made.

1. The present findings should be validated using compressed and uncompressed dairy products.

2. Experiments should be undertaken to determine the effect of water activity and temperature upon the acetyllysine-glucose-cellulose model. A long-term study at 40°C would be particularly relevant to the unrefrigerated storage stability of NSM foods.

3. Similar studies should be conducted using protein-reducing carbohydrate systems.

4. The kinetics of the acetyllysine-glucose reaction should be determined in a homogenous liquid medium to correct for the inherent defects of the solid system. Whether the reaction is of a predictable order in solution or whether the concept of reaction order does not apply to the NAL-GL reaction should be established.

REFERENCES

1. MAURON, J. The Maillard reaction in food; A critical review from the nutritional standpoint. In Progress in Food and Nutrition Science. Maillard Reactions in Food, (C. Ericksson, Ed.) Pergammon Press Ltd., Great Britain, 1981, pp 5-35.

2. HURREL, R.F. and CARPENTER, K.J. The estimation of available lysine in food stuffs after Maillard reactions. In Progress in Food and Nutrition Science. Maillard Reactions in Food, (C. Eriksson, Ed.) Pergammon Press Ltd., Great Britain, 1981, pp 159-176.

3. U.S. Army Natick Research and Development Center, Annual Historical Review, U. S. Army Natick Research and Development Center, 1983, p 43.

4. CHIANG, G.H. A simple and rapid high performance liquid chromatographic procedure for determination of furosine, lysine-reducing sugar derivative. J. Agric. Food Chem. 31:1373, 1983.

5. CROWE, L.K., JENNESS, R. and COULTER, S.T. The reducing capacity of milk and milk products as measured by a modified ferricyanide method. J. Dairy Sci. 31:595, 1948.

6. BIDLINGMEYER, B.A., COHEN, S.A. and TARVIN, T.L. Rapid analysis of amino acids using pre-column derivatization. J. Chromatog. 336:93, 1984.

7. NARAYAN, K.A. and ANDREOTTI, R. Safeguarding the nutritional adequacy of Nutritional Sustainment Modules: Kinetics of quality loss due to Maillard reaction. In Natick Research Development & Engineering Center, Science Symposium Proceedings, 2-4 June 1986, Volume I, Technical Report, NATICK/TR-86/050, 1986, pp 133-174.

8. NARAYAN, K.A. and ANDREOTTI, R. Protein quality deterioration in compressed models. Fed. Proc. 45:1093, 1986.

9. LABUZA, T.P. and SALTMARCH, M. The nonenzymatic browning reaction as affected by water in foods. In Water Activity/Influences on Food Quality (L.B. Rockland and G.F. Stewart, Eds.), 1981, pp 630-650.

10. WOLF, J.C., THOMPSON, D.R. and REINECCIUS, G.A. Initial losses of available lysine in model systems. J. Food Sci., 42:1540, 1977.